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Cyanobacterial toxins in Italian freshwaters

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Abstract

This study focuses on the occurrence and distribution of cyanobacterial toxins from 1989 to 2006 in several Italian lakes of different characteristics and human uses, the latter including drinking water abstraction and recreation. Phytoplankton and LC/MS/MS toxin analyses were performed on surface water samples collected from 28 lakes. The most widespread species associated with toxin production belonged to the genera *Microcystis*, *Planktothrix* and *Anabaena*. Extracellular concentrations varied from non-detectable values up to 226.16 ng/mL for microcystins (sum of all variants), to 126 ng/mL for total cylindrospermopsin, and to 100 μg/g (dry weight) for anatoxin-a.

The toxin concentrations in the lake waters did not always correlate with the cyanobacteria cell densities. This implies a need for control studies including toxin detection in water together with microscopic cell evaluations, in order to avoid possible toxin underestimates.
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Keywords: Cyanobacteria; Cyanotoxins; Microcystin; Cylindrospermopsin; Anatoxin-a; Freshwaters; Lakes; Italy

Introduction

Cyanobacteria are a widespread group of organisms colonizing all ecosystems. They are common inhabitants of freshwater bodies throughout the world and several of them form surface scums (blooms). Under favourable conditions several species of cyanobacteria may become dominant in the phytoplankton of water bodies. Cell densities may reach many millions per litre (Chorus and Bartram, 1999).

Cyanobacteria are known to produce several metabolites significant from the public health perspective of acute exposure: lipopolysaccharides (Stewart et al., 2006), and cytotoxic, tumor-promoting and enzyme-inhibiting metabolites like cyclic depsipeptides, cyclic peptides (anabaenopeptins and nostophycins), linear peptides (aeruginosins and microginins) (Bickel et al., 2001; Forchert et al., 2001; Welker and Von Dohren, 2006). Recently, a neurotoxic non-protein aminoacid

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(*N*-methylamino-L-alanine, BMAA), widely produced among cyanobacteria (Cox et al., 2005), has been associated with neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis/parkinsonism-dementia complex (Murch et al., 2004).

Many cyanobacterial species can produce several categories of powerful toxins that are unique to this group of organisms, with the exception of saxitoxins. Cyanotoxin poisoning in humans was mainly caused by three toxic groups: microcystins (MCYSTs), cylindrospermopsin and anatoxin-a (ANA-a), and occurred through exposure to contaminated drinking water supplies (Annadotter et al., 2001; Falconer, 2005), recreational waters (Chorus and Bartram, 1999; Behm, 2003), medical dialysis (Azevedo et al., 2002).

Most poisoning by cyanobacteria involves acute hepatotoxicosis caused by a structurally similar group of small molecular weight cyclic hepta-peptides, the microcystins. MCYSTs are genotoxic (Bouaicha et al., 2005), protein phosphatase-inhibiting (Dawson, 1998) toxins responsible for liver failure and death in humans (Azevedo et al., 2002), wild animals, livestock and aquatic life (Sivonen and Jones, 1999; Mwaura et al., 2004). Over ninety MCYSTs variants are now described (Welker and Von Dohren, 2006), produced by a number of species of not more than ten cyanobacterial genera. Indirect evidence supporting tumour promotion of human cancer from MCYST exposure comes from the studies of Yu (1989), Ueno et al. (1996) and Zhou et al. (2002) in China, and Fleming et al. (2002) in Florida.

Poisoning episodes are also caused by cylindrospermopsin (CYN), which is a nephrotoxic, thymotoxic and hepatotoxic cyclic guanidine alkaloid (Terao et al., 1994). Only recently, mutagenicity of CYN was shown *in vitro* and strong evidence also exists for its carcinogenicity *in vivo* (Humpage et al., 2000; Shen et al., 2002).

ANA-a is a potent neurotoxic alkaloid first detected in the cyanobacterium *Anabaena flos-aquae* Brébisson ex Bornet et Flahault, and it is perhaps one of the most powerful cyanobacterial toxin (Carmichael, 1994). Poisoning episodes by this toxin increased in severity in recent years (Behm, 2003).

A few data are available in literature on the European diffusion of the latter two toxin groups (Rücker et al., 2007; Sivonen et al., 1989; Edwards et al., 1992; James et al., 1997; Bunke-Vogt et al., 1999; Pawlik-Skowronska et al., 2004), as well as studies on the occurrence and diffusion of cyanotoxins in Italian water bodies (Bruno et al., 1989, 1992, 1994; Pomati et al., 2000; Viaggiu et al., 2004; Manti et al., 2005; Messineo et al., 2006; Naselli-Flores et al., 2007).

In 1985, for the first time, suspected toxic algal blooms occurring in the Italian artificial lakes of Medio Flumendosa and Mulargia (Sardinia, western Mediterranean Sea) were investigated by our laboratory. Some analyses on these blooms of *Planktothrix rubescens* ex *Oscillatoria rubescens* (D.C. ex Gomont) Komarek & Anagnostidis evidenced the presence of an hepatotoxic substance, with a LD50 of 100 mg/Kg body weight in mice and with MCYST characteristics (considerable liver damages, pulmonary and kidney haemorrhages) (Loizzo et al., 1988).

Since then, reports of toxic blooms continually reached our laboratory, and in 1993 a data bank concerning both marine and freshwater algal bloom episodes was set up. The presence of cyanobacterial blooms in drinking and recreational waters required surveys on their occurrence and presence of toxins, in order to avoid human health risks and to provide information for successful restoration programs.

The present study focuses on the occurrence and distribution of cyanobacterial toxins from 1989 to 2006 in several Italian lakes different for characteristics and human uses.

Materials and methods

Samples collection

Surface water samples for chemical analyses including cyanotoxins, and for biological investigations of cyanobacteria were collected between 1989 and 2006 from 28 Italian lakes (Fig. 1), all used for drinking-water abstraction and/or recreation (Table 1), from stations situated close to the lake shore. Sampling stations 500 m from the dams were adopted for all the Sardinian lakes. Subsamples (100 mL) were immediately fixed with Lugol's solution for the microscopic evaluations of cell densities. Samples were stored in ice chests and transported in dark and refrigerated conditions to the laboratory where toxin analyses were performed.

Reagents and chemicals

MC-RR, MC-YR, MC-LR, MC-LA, MC-LW were purchased by Calbiochem, La Jolla, CA. ANA-a and CYN were from Sigma-Aldrich, Milwaukee, WI. Trimethacarb (Riedel-de Haën, Seelze, Germany) is an obsolete insecticide and was used as internal standard (IS) for quantifying MCYSTs, while quantification of CYN was carried out using 1,9-diaminononane (Sigma-Aldrich) as IS.

Individual standard solutions of the five MCYSTs, ANA-a and CYN were prepared by dissolving each compound in water to obtain 25 μg/mL concentrations. After preparation, these solutions were stored at −18 °C in the dark to minimize analyte degradation. They were freshly prepared every 2 months. A composite working standard solution of MCYSTs was prepared by mixing the above solutions and diluting with water to obtain

Fig. 1. Geographical localisation of the 28 sampled water bodies.

analyte concentrations of 1 μg/mL. One milligram per milliliter solutions of the two ISs, that is trimethacarb and 1,9-diaminononane, were separately prepared by dissolving them in acetonitrile. We obtained distinct working solutions of the two ISs at 1.5 μg/mL concentration by diluting with 10 mmol/L formic acid-acidified acetonitrile. When unused, all working standard solutions were stored at 4 °C in the dark, and renewed after one working week.

Acetonitrile RS of gradient grade was obtained from Carlo Erba, Milan, Italy. Trifluoroacetic acid (TFA) was from Fluka Bucks, Switzerland. All other solvents and chemicals were of analytical grade (Carlo Erba), and were used as supplied.

Phytoplankton analysis

The microscopic analyses of phytoplankton were carried out on fresh and Lugol fixed samples in 25/10/5 mL sedimentation chambers, using an inverted microscope (Leitz Labovert FS; Zeiss Axiovert 100). Cell density was determined according to Utermöhl (1931) and Lund et al. (1958). Species were determined on both fresh and fixed samples according to Anagnostidis and Komarek (1990), Komarek and Anagnostidis (1989, 2005) and Cronberg and Annadotter (2006).

Toxin extraction

Samples transported in laboratory were immediately analyzed for toxin content. Toxins were extracted from seston following the procedure described by Meriluoto and Eriksson (1988). Fresh seston aliquots (10–50 mg) were obtained by centrifugation of water samples (11000 rpm, Beckman LT-55 Ultracentrifuge) in Eppendorf vials. The water supernatant was completely aspired by a Gilson micropipette, the pellet briefly air-dried under a cold air-flow in order to minimize the residual water content, and then weighted (wet weight).

Table 1. Investigated water bodies, main characteristics and uses

Name	Type of water body	Area (km^2)	Max. depth (m)	Use**
Iseo	Glacial lake	61	250.7	D-R
Albano	Volcanic lake	6.02	170	R
Mulargia	Reservoir	13	94	D-R
Gusana	Reservoir	2.52	79.5	D-R
Fiastrone	Reservoir	0.89	78.5	D-R
Liscia	Reservoir	5.6	63.5	D
Monteleone	Reservoir	4.81	60	D
Liscione	Reservoir	7.45	57	D-R
Flumendosa	Reservoir	3.25	55	D
Vico	Volcanic lake	12.09	49.5	*D-R*
Cecita	Reservoir	10.41	46	R
Gerosa	Reservoir	0.72	45	D-R
Cucchinadorza	Reservoir	1.19	43	D
Torrei	Reservoir	0.09	38.5	D
Nemi	Volcanic lake	1.67	34	R
Bidighinzu	Reservoir	1.5	34	D
S. Puoto	Volcanic lake	0.3	32	R
Canterno	Carsic lake	0.65	30	R
Posada	Reservoir	3	29.5	D
Govossai	Reservoir	0.5	28.1	D
Cedrino	Reservoir	1.5	26.5 #	D
Polverina	Reservoir	0.74	23	R
Simbirizzi	Reservoir	3.2	16.5	R
Benzone	Reservoir	0.28	15.5	D
Pattada	Reservoir	4.27	14.9 #	D
Spino	Fishing pond	0.013	10	R
Trasimeno	Tectonic lake	128	6	D-R
Massaciuccoli	Coastal lake	7	3	R

**D = drinking; R = recreational.
= mean depth.

Before year 1992, the samples (1 L) were lyophilized and stored at −30 °C (dry weight).

Compared to the higher dry weight derived toxin values, the wet weight derived values may be defined as minimum seston toxin contents, and in this sense our values can be considered.

The centrifuged cells were suspended in 1 mL sterile bidistilled H_2O to perform toxin extraction. The solution was stirred, ultrasonicated (5 min at 30–40 °C) (Vibra-Cell, Sonics & Materials Inc.), then centrifuged for 10 min at 11000 rpm (Beckman, LT-55 Ultracentrifuge) to eliminate debris. The supernatant was collected, and the whole process repeated twice. Supernatants were then pooled and stored at −30 °C for analyses.

At the same time, water sample aliquots (0.5 L) were filtered on 0.45 μm cellulose nitrate filters (Millipore, Inc.), and extracellular MCYST concentrations were determined from the filtrate water.

Water samples extraction

Water sample extraction was performed according to a method described by Bogialli et al. (2006).

Briefly, MCYSTs were extracted from 0.5 L of lake water passing the sample through a cartridge filled with 0.5 g Carbograph 4, while CYN was directly injected to LC/MS/MS apparatus after filtration. MCYST elution was done in back-flushing mode with 1 mL of methanol followed by 4 mL of 10 mmol/L TFA-acidified CH_2Cl_2/CH_3OH (80:20, v/v). After adding IS the eluate was evaporated to about 50 μL in a water bath at 50 °C, 200 μL of a mobile phase (see below) was added to the residue and 50 μL of the final extract was injected into the LC column.

Instrumental analysis

Cyanotoxins detected before year 2000 were analyzed by HPLC-DAD (MCYSTs) using the only three available commercial standards (MC-LR, MC-RR, MC-YR), or by GC-MS (ANA-a) (Bruno et al., 1994); so different MCYST variants, if present, could not be detected. Since 2000 more MCYST standards (MC-LA, MC-LW) were available, analyses were performed by LC-MS/MS and on the basis of the higher accuracy of

the data analyses, the samples of this last group of years were examined also for some MCYST isomer presence.

LC/MS/MS analysis, quantitation and limits of quantification (LOQs)

LC/MS/MS analysis was performed according to the method described by Bogialli et al. (2006). By following the method described by Hummert et al. (2001), two demethylated varieties of MC-RR and one demethylated form of MC-LR were identified on monitoring cyanotoxins in Lake Albano. Standards of these three MCYSTs were not available to us. Therefore, a semi-quantitative estimation of the concentrations of the above cited MCYSTs was done by assigning to them the same molar response factors of the respective fully methylated MCYSTs. LOQs were then calculated on the basis of a minimal accepted value of the signal-to-noise ratio (S/N) of 10. LOQs of MCYSTs were between 2 (MC-RR) and 9 (MC-LW) ng/L. In spite of the fact that no enrichment step of CYN could be performed by the SPE cartridge, LOQ of this toxin was estimated to be 300 ng/L (Bogialli et al., 2006).

Quantification

Analytes for which standards were available were quantified by the external standard quantification procedure. Standard solutions were prepared at eight levels by using appropriate volumes of the working standard solution. For each analyte, the peak area versus injected amount chart was obtained by measuring at any injected amount the resulting peak area and relating this area to that of the internal standard. The response of the ESI-MS/MS system was linearly related to injected amounts of the analytes up to 300 ng. When amounts of MCs injected from extracts of lake water samples exceeded the upper limit of the linear dynamic range of the detector response, extracts were suitably diluted and re-injected.

Results and discussion

Cyanobacterial hepatotoxins (MCYST and CYN) and the neurotoxin ANA-a were detected in 87 surface water and scum samples from the 28 lakes studied, collected in the occurrence of algal blooms. Almost all samples positive for cyanotoxins showed the contemporary presence of Cyanobacteria. Only in a very few cases the microscopic observation did not assess Cyanobacteria cells or species already known as toxin producers. In the cases of Sardinia lakes Benzone (December 2004), Govossai (December 2004) and Gusana (December 2004), the samples were collected just after dense and prolonged blooms (from summer to autumn) of species probably toxic (*Microcystis* spp.). The toxins detected are likely to have originated from these events, which were not analyzed for toxin occurrence.

Cyanobacteria total densities ranged from less than 10^6 to more than 200×10^9 cells/L. The dominant species (>70% of the cyanobacterial total density of each sample) belonged to 11 genera: *Anabaena*, *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*, *Cylindrospermopsis*, *Lyngbya,* *Merismopedia*, *Microcystis,* *Oscillatoria,* *Planktothrix*, *Pseudanabaena*, *Woronichia*. Blooms were generally dominated by one or two species. *Planktothrix* (mainly *P. rubescens*) and *Microcystis* blooms (mainly *M. aeruginosa* (Kutzing) Lemmermann) dominated in the samples from 33% of the lakes, respectively. The other lakes presented blooms caused by the assemblages of two or more co-dominating cyanobacterial genera (Figs. 2 and 3). In the continental part of Italy, *P. rubescens* was the dominant blooming and MCYST-producing cyanobacterium, followed by *M. aeruginosa* whereas in Sardinian Island the latter was dominant. In lakes Massaciuccoli, Trasimeno, Cecita, Liscione and Polverina, positive for MCYSTs, *M. aeruginosa* blooms were present, but cell densities were not quantified.

Six MCYST variants, desmethyl MC-RR ([Dha^7] MC-RR or [D-Asp^3, (E)-Dhb^7] MC-RR), MC-RR, MC-YR, MC-LR, MC-LA and MC-LW were detected in the samples. Most samples analyzed contained more than three MCYSTs, being demethylated MCYSTs, MC-RR, MC-LR and MC-YR the main constituents.

Total MCYST concentration in superficial scums varied between 107 (Iseo Lake, wet weight) and 1160×10^3 ng/g (San Puoto Lake, dry weight).

In Albano Lake, the cell density related to the analyzed surface scums led to a total intracellular MCYST concentration/L ranging from 84 μg/L to 10.1 mg/L.

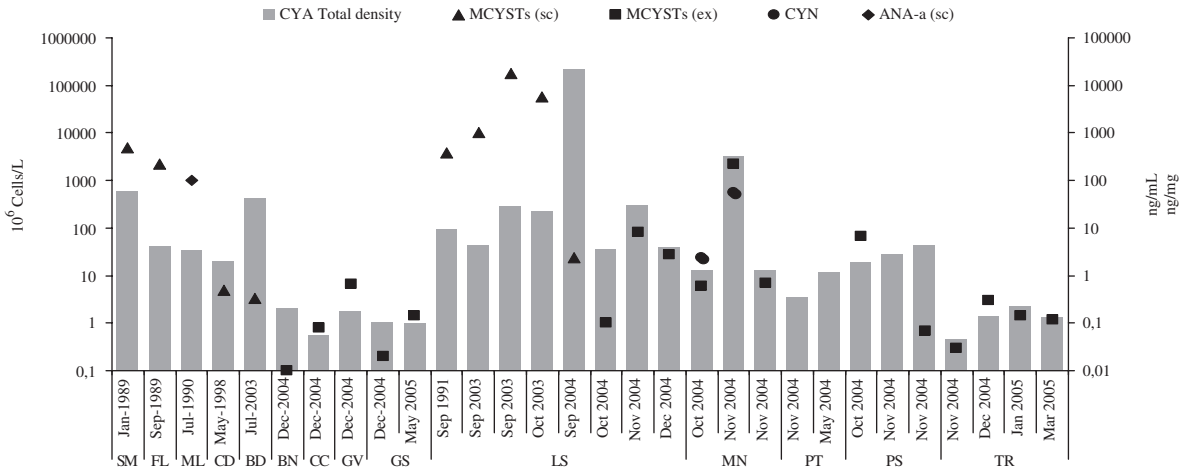
The proportion of dissolved toxin compared to total toxin concentration ranged from 0.003% to 1.8% in these superficial blooms.

The scums formed by *Planktothrix* blooms showed the highest MC-RR and MC-RR plus desmethyl MC-RR amounts in Simbirizzi Lake (480×10^3 ng/g MC-RR, dry weight) and Albano Lake (198.8×10^3 ng/g, wet weight) (Figs. 2 and 3); the highest concentration of MC-YR (1160×10^3 ng/g, dry weight) in San Puoto Lake, and the highest value of MC-LR (80.3×10^3 ng/g, wet weight) in a scum from Albano Lake.

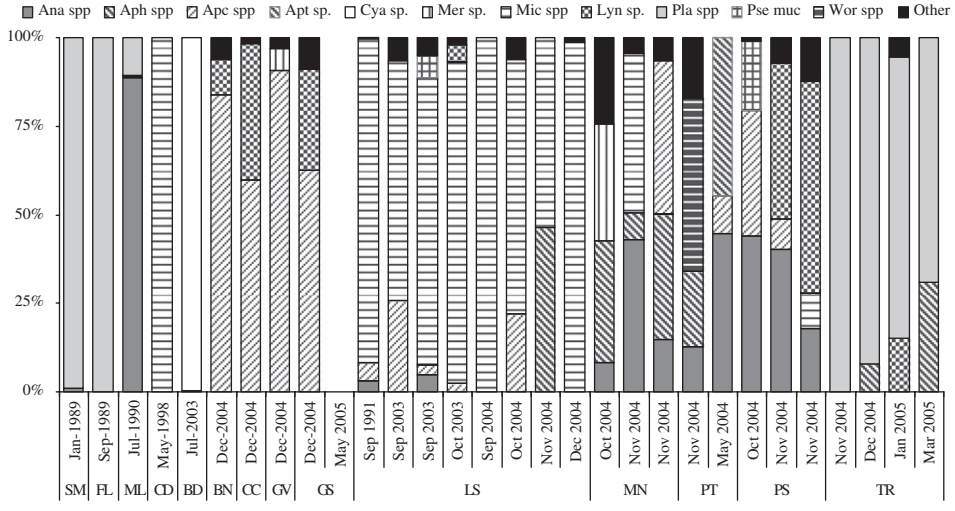
In the scum samples from *Microcystis* blooms the highest MC-RR plus desmethyl MC-RR amount was detected in Trasimeno Lake (39×10^3 ng/g, wet weight); the highest concentrations of MC-YR (150×10^3 ng/g, wet weight) and of MC-LR (380×10^3 ng/g, dry weight) were found in Massaciuccoli Lake and Liscia Lake,

respectively. In the superficial scums desmethyl MC-RR plus MC-RR and MC-YR were generally predominant (from 32% to 100% and from 2% to 100%, respectively; analyses subsequent to year 2000).

Extracellular MCYSTs in water were detected in 51 samples from 18 lakes, and ranged from traces (minor than 0.004 ng/ml) to 226.16 ng/ml (total extracellular concentration, Figs. 2 and 3).



(a)



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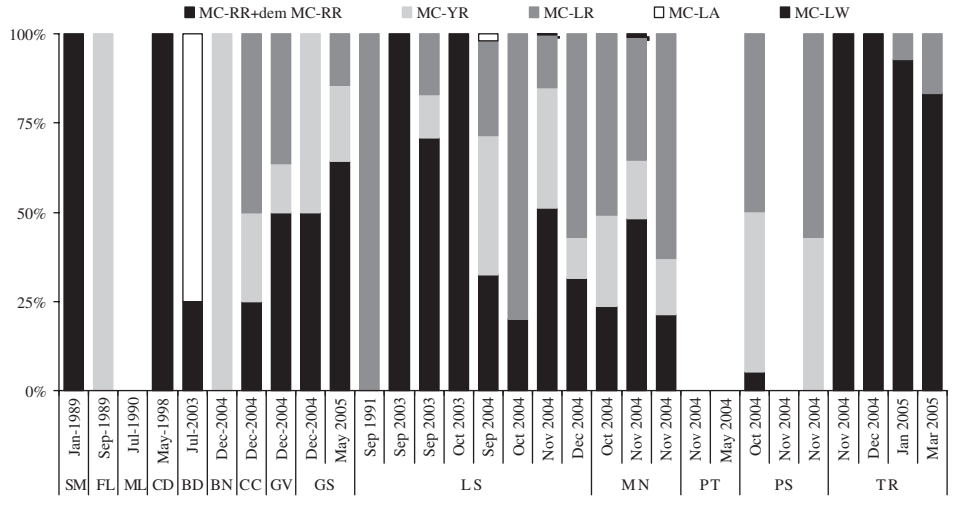

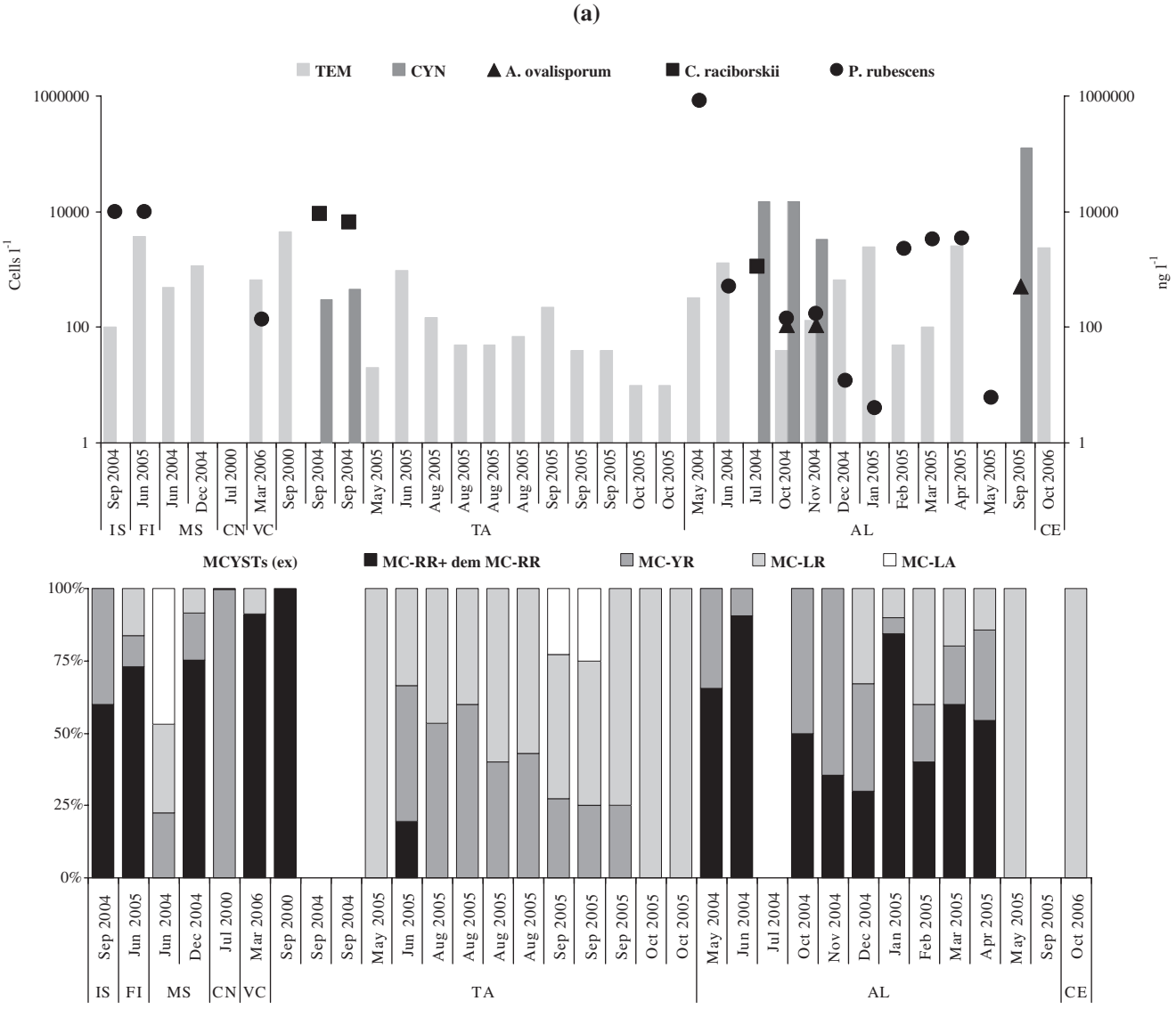

Fig. 3. Peninsular Italian lakes: (a) total cyanobacterial cell density and cyanotoxin concentrations in extracellular water contents (upper graphic) and related composition of total extracellular microcystins by structural variants (lower graphic); (b) total cyanobacterial cell density and cyanotoxin concentrations in scums (upper graphic) and related composition of total intracellular microcystins by structural variants (lower graphic). *Lake abbreviations*: AL = Albano; CN = Canterno; CE = Cecita; FI = Fiastrone; GE = Gerosa; IS = Iseo; LI = Liscione; MS = Massaciuccoli; NE = Nemi; PL = Polverina; S. P = S. Puoto; SI = Spino; TA = Trasimeno; VC = Vico;. *Toxin abbreviations*: MCYSTs (sc) = total microcystin concentrations in scums; MCYSTs (ex) = total extracellular microcystin concentrations; CYN = cylindrospermopsin concentrations

Fig. 2. Sardinian reservoirs: total cyanobacterial cell density and cyanotoxin concentrations (upper graphic), related species composition of the Cyanobacteria assemblages in % of total cyanobacterial cell counts (middle graphic) and composition of total extracellular microcystins by structural variants (lower graphic). *Lake abbreviations*: SM = Simbirizzi; FL = Flumendosa; ML = Mulargia; CD = Cedrino; BD = Bidighinzu; BN = Benzone; CC = Cucchinadorza; GV = Govossai; LS = Liscia; MN = Monteleone; PT = Pattada; PS = Posada; TR = Torrei. *Toxin abbreviations*: MCYSTs (sc) = total microcystin concentrations in scums; MCYSTs (ex) = total extracellular microcystin concentrations; CYN = cylindrospermopsin concentrations; ANA-a = anatoxin-a concentrations. *Species abbreviations*: Ana spp = Anabaena planctonica, A. spiroides, A. flos-aquae, Anabaena sp.; Aph spp = Aphanizomenon flos-aquae, Aph. ovalisporum; Apc spp = Aphanocapsa incerta, Aphanocapsa spp.; Apt spp = Aphanothece spp.; Mer sp. = Merismopedia sp.; Mic spp = Microcystis aeruginosa, M. viridis, M. flos-aquae; Lyn sp. = Lyngbya sp.; Pla spp = Planktothrix ruscens, Planktothrix sp.; Pse muc = Pseudanabaena mucicola; Wor spp = Woronichinia naegeliana, Woronichinia sp.

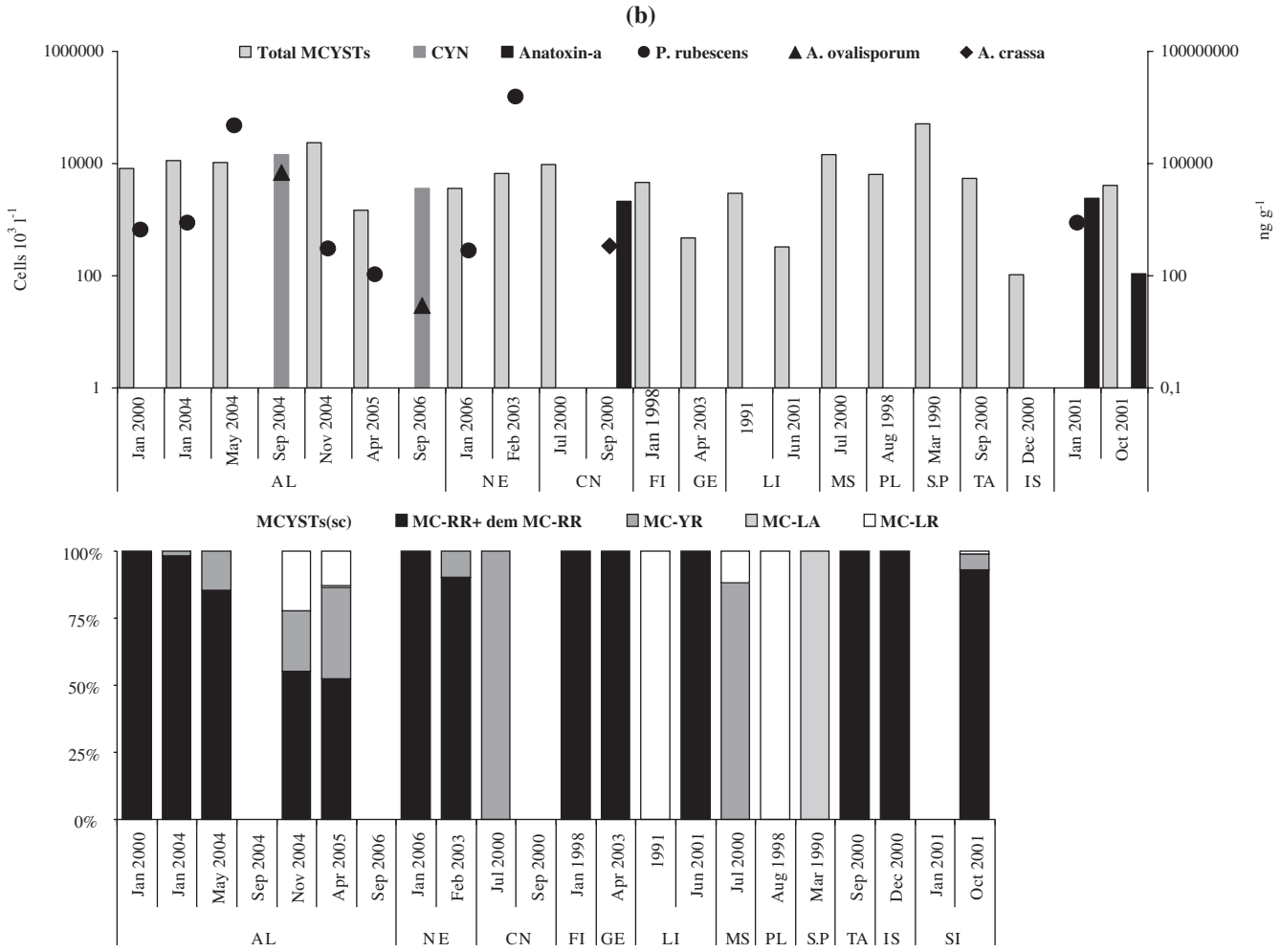

Fig. 3. (*Continued*)

The three highest values of extracellular total MCYSTs in water (226.16, 8.32 and 6.77 ng/ml) were found in Monteleone, Liscia and Posada lakes in November 2004 and October 2004, respectively.

Twelve out of the 51 samples (23%, range: 0.98–226.16 ng/ml), exceeded the WHO provisional value of 1 μg/L (Kuiper-Goodman et al., 1999) and the Italian governmental limit for freshwater bathing waters (0.84 μg/L or 5000 cell of cyanobacterial toxic species/mL, Health Min. Circ. 31/7/1998 IX.400.4/13.1/3/1447). The percentage increases if the limits suggested by other studies are considered (0.1 μg/L, Annadotter et al., 2001; 0.01 μg/L, Ueno et al., 1996).

LC-MS/MS analyses showed desmethyl MC-RR plus MC-RR (being desmethyl MC-RR the main constituent) and MC-YR as the predominant MCYST variants in the extracellular concentrations of *Planktothrix* dominated lakes (from 30% to 100% and from 5% to 50%, respectively) while MC-LR and MC-YR were the predominant toxins in *Microcystis* dominated lakes (from 8% to 100% and from 10% to 100%, respectively). MC-LW and MC-LA extracellular concentrations in surface water were almost always under detection limits, with maxima in Massaciuccoli Lake (0.23 ng/mL MC-LA, June 2004), and in Monteleone Lake (2.27 ng/mL MC-LW, November 2004). MC-LA was rarely detected in the Italian samples, as it was described in natural blooms of other countries (South Africa, Scott, 1991; Morocco, Oudra et al., 2001; Greece, Gkelis et al., 2005).

The total MCYST concentration in superficial scums in Italy is comparable with other European countries (Poland, 23–1687 μg/g, Jurczak et al., 2004; Denmark, 11–737 μg/g, Henriksen and Moestrup, 1997) and with concentrations detected in surface waters from various other continents (from few ng/L up to 1300 μg/L; Fromme et al., 2000). Comparison of our data showed lower MCYST contents/g in *Microcystis* dominated lakes than in *Planktothrix* dominated lakes, as observed by other authors (Willame et al., 2005). Demethylated MCYSTs were in general the predominant toxins, as Fastner et al. (1999) already observed in German freshwaters ; they were always detected in *Planktothrix* blooms (being (D-asp^3,

(E)-Dhb^7 or Dha^7) the most abundant), and in several *Microcystis* blooms.

Although not unusual in eutrophic lakes, the high extracellular values detected are often in the high end of the reported ranges (Sivonen and Jones, 1999); in the case of extended blooms which intracellular contents were not analyzed (i.e. in Monteleone Lake, November 2004), the related high levels of extracellular MCYSTs detected could be reputed commensurate.

Extracellular CYN concentrations in surface water ranged from 0.3 (Trasimeno Lake, September 2004) to 126 ng/mL (Albano Lake, September 2005) (Figs. 2 and 3). CYN detection was always connected with blooms dominated or co-dominated by *Aphanizomenon ovalisporum* Forti or *Cylindrospermopsis raciborskii* (Woloszinska) Seenaya & Subba Raju species. The two highest values were observed in Albano Lake (see above) and in Monteleone Lake (56.3 ng/mL, November 2004). Even if in the range of occurring values reported by Rücker et al. (2007), all the extracellular concentrations exceeded the recommended limit of 1 μg/L (Humpage and Falconer, 2003), except for Trasimeno Lake. Albano Lake showed concentrations of 21.1 (September 2006) and 175.1 (September 2004) ng/mg intracellular CYN in wet weight samples from two scums of *Aph. ovalisporum* (Fig. 3).

The detection of CYN in our samples was less frequent than MCYSTs. It might be due to a lack of specific surveys, or to a narrow distribution of the species *C. raciborskii* and *Aph. ovalisporum* to which, in Italy, the toxin appears to be solely connected, in contrast with observations in other Countries (Spoof et al., 2006; Preußel et al., 2006). The presence of this toxin was for the first time detected in Europe in 2002 (Kiss et al., 2002) and in Italy in 2004 (Manti et al., 2005).

ANA-a concentrations in the analyzed scums were from 115.1 ng/g (October 2001) to 12.13 μg/g (January 2001) in Spino Lake, as wet weight, and 100 μg/g (July 1990) in Mulargia Lake, as dry weight, the latter value found during an *Anabaena planctonica* Brunnth. bloom (Fig. 2). *A. planctonica* bloom in Mulargia Lake showed also the presence of not identified MCYST – like peptides (Bruno et al. 1994), which may give reason for the MCYST amounts in October and December 2004 samples from Posada Lake, where presence of *A. planctonica* blooms was detected. ANA-a detection in Spino Lake was due to the presence of a peculiar *P. rubescens* population, MCYST producer, too (Viaggiu et al. 2004). ANA-a was also associated to a bloom of *Anabaena crassa* (Lemmermann) Komárkova-Legnerová et Cronberg in Canterno Lake (9.8 μg/g, September 2000).

The ANA-a values lie in the mid-low range reported by Sivonen and Jones (1999), but due to the low number of positive analyzed samples (four samples from three lakes), more extended and focused studies are needed to define the real presence of this toxin in Italy.

Considering CYN and MCYST extracellular contents, the toxin contents detected in waters often did not correlate with the cyanobacterial cell density (Figs. 2 and 3), according to observations in previous studies (Codd, 1995; Shaw et al., 1999; Messineo et al., 2006). This lack of correlation could be expected considering the wide range of involved taxa and variety of water bodies, but it could also depend on the phase of the bloom: samplings carried out in the period of decline, with the lowering of cell numbers and the corresponding release of toxins in waters during cell lysis, could account for the extracellular MCYST levels, as observed by Park et al. (1998).

Large proportions of CYN can be present extracellularly, and might persist for long periods (Chiswell et al., 1999; Saker and Griffiths, 2000; Mc Gregor and Fabbro, 2000; Metcalf et al., 2002). The observation seems to be confirmed in Albano Lake samples, but further investigations are needed on more extended sample series and on the dynamics of the producer species.

The evidence that toxin contents may not correlate with the cyanobacterial cell density suggests monitoring based on total toxin detections in water together with microscopic cell count, instead of only the latter, as it is practiced in most Italian Territorial Laboratories, to avoid possible under- or overestimates of the real toxin levels present in the waters.

More extended environmental and epidemiological studies are needed to define the risk assessment related to toxic eutrophication events in Italy, but some possible major routes of human exposure may be considered: the presence of superficial scums during the bathing season (June–September) as in the case of Albano Lake, the consumption of contaminated ichthyic fauna (Bogialli et al., 2005), and the use of affected lakes and reservoirs or related groundwaters as the only drinking water supply by many Italian towns.

The high extracellular MCYST and CYN levels detected can constitute a serious risk factor for drinking water consumers, due to the inability of conventional water treatment plans to remove them. Moreover, surface water data should be considered with those on groundwater contamination. In Italian studies, MCYSTs were detected in two wells for human use near Lake Albano (Messineo et al., 2006), and in ten wells for human use near Lake Vico.

Cyanobacterial dominance is widespread in Italian lakes (Salmaso et al., 1994; Salmaso, 2000) and in reservoirs of Southern Italy such as in Sardinia (Sechi and Lugliè, 1992, 1996), Calabria and Sicily regions (Barone et al., 1991). These findings are consistent with high summer temperatures and strong thermal stratification of these reservoirs, despite their relative shallowness,

and with the very frequent eutrophication of these freshwater environments (Marchetti et al., 1992). However, a few studies on cyanotoxin detection have been performed, on single or little groups of lakes.

Our study shows for the first time the extended occurrence of a wide spectrum of cyanotoxins in many Italian lakes as a consequence of cyanobacterial dominance, sometimes with high-extracellular toxin levels reached. In several regions was detected the presence of two toxins, CYN and ANA-a, which diffusion in Europe is still little investigated.

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